Data Evaluation Report on the Acute Toxicity of Fluopyram & Trifloxystrobin SC 500 (250+250) G (AIs: fluopyram and trifloxystrobin) to Fish (*Oncorhynchus mykiss*)

PMRA Submission Number {.....} EPA MRID Number 47567514

Data Requirement:

PMRA Data Code	{.....}
EPA DP Barcode	360307
OECD Data Point	{.....}
EPA MRID	47567514
EPA Guideline	850.1075

Test material: **AE C656948 &Trifloxystrobin SC 250 + 250 G (AIs: fluopyram and trifloxystrobin)**

AI: Fluopyram Purity: 21.4%

Common name

Chemical name: IUPAC N-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS name N-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)benzamide
CAS No. 658066-35-4
Synonyms AE C656948

AI: Trifloxystrobin Purity: 21.6%

Common name

Chemical name: IUPAC methyl (E)-methoxyimino-{(E)-α-[1-(α,α,α-trifluoro-m-tolyl)ethylideneaminooxy]-o-tolyl}acetate
CAS name methyl (α*E*)-α-(methoxyimino)-2-[[[[(1*E*)-1-[3-(trifluoromethyl)phenyl]ethylidene]amino]oxy]methyl]benzeneacetate
CAS No. 141517-21-7
Synonyms CGA 279202

Primary Reviewer: Moncie Wright
Staff Scientist, Cambridge Environmental

Moncie V Wright
Signature:
Date: 12/10/09

Secondary Reviewer: Teri S. Myers
Senior Scientist, Cambridge Environmental Inc.

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Date: 01/04/10

Primary Reviewer: F. Nicholas Mastrota
Wildlife Biologist
Environmental Fate and Effects Division
US Environmental Protection Agency

Date: 3/25/2011

EPA PC Code 129112 and 080302

Date Evaluation Completed: 25-03-2011

CITATION: Dorgerloh, M. 2007. Acute toxicity of fluopyram & trifloxystrobin SC 500 (250+250) G to fish (*Oncorhynchus mykiss*) under static conditions. Unpublished study performed and sponsored by Bayer CropScience AG, Monheim, Germany. Laboratory Project ID: E 280 3307-3. Report ID: EBGMP030. Study completed November 5, 2007.

EXECUTIVE SUMMARY:

In a 96-h acute toxicity study, rainbow trout (*Oncorhynchus mykiss*) were exposed to **AE C656948 &Trifloxystrobin SC 250 + 250 G (AIs: fluopyram and trifloxystrobin)** at nominal concentrations of 0 (negative

control), 31.3, 62.5, 125, 250, and 500 µg form/L, which were equivalent to nominal concentrations of 0 (negative control), 6.76, 13.5, 27.0, 54.0, and 108 µg trifloxystrobin/L. Mean-measured concentrations of fluopyram were <0.521 (<LSC; negative control), 7.26, 13.6, 26.1, 53.1, and 103 µg fluopyram/L. The test was conducted under static conditions.

The NOAEC (based on mortality and sublethal effects) and LC_{50} values were 62.5 and 88.4 µg form/L, which were equivalent to 13.6 and 18.8 µg fluopyram/L, and 13.5 and 19.1 µg trifloxystrobin/L.

Sublethal effects such as inactivity/low activity, labored respiration, lying on the bottom of the aquarium, weak coloration, lying on their sides or backs, and loss of equilibrium with lateral deviation were observed in the groups exposed to the 125 and 250 µg form/L treatment groups. Complete mortality in the 500 µg form/L treatment group precluded any observations.

Based on the results of this study, **AE C656948 &Trifloxystrobin SC 250 + 250 G** would be classified as very highly toxic to rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis, in accordance with the classification system of the U.S. EPA.

This toxicity study is classified as **acceptable**. It satisfies the guideline requirement for an acute toxicity with freshwater fish (cold water species) study with this product.

Results Synopsis

Test Organism Size/Age(mean weight or length): 1.6 ± 0.6 g; age not reported
Test Type (Flow-through, Static, Static Renewal): Static

Formulation (nominal)
LC_{50}: 88.4 µg form/L 95% C.I.: 62.5 to 125 µg form/L
NOAEC: 62.5 µg form/L (based on mortality and sublethal effects)
Probit Slope: N/A 95% C.I.: N/A

Fluopyram (mean-measured)
LC_{50}: 18.8 µg fluopyram/L 95% C.I.: 13.6 to 26.1 µg fluopyram /L
NOAEC: 13.6 µg fluopyram/L (based on mortality and sublethal effects)
Probit Slope: N/A 95% C.I.: N/A

Trifloxystrobin (nominal)
LC_{50}: 19.1 µg trifloxystrobin/L 95% C.I.: 13.5 to 27.0 µg trifloxystrobin/L
NOAEC: 13.5 µg trifloxystrobin/L (based on mortality and sublethal effects)
Probit Slope: N/A 95% C.I.: N/A

Endpoints affected: mortality and sublethal effects

Sublethal effects: inactivity/low activity, labored respiration, lying on the bottom of the aquarium, weak coloration, lying on their sides or backs, and loss of equilibrium with lateral deviation from normal orientation

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PMRA Submission Number {.....} EPA MRID Number 47567514

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted according to the following guidelines: EPA FIFRA § 72-1, Acute Toxicity Test for Freshwater Fish/SEP-EPA-540/9-85-006 (1982/1985); OCSPP 850.1075, Fish Acute Toxicity Test, Freshwater and Marine (public draft; 1996); EU Commission Directive 92/69/EEC, C.1: Acute Toxicity for Fish (1992); and OECD Guideline for Testing of Chemicals, No. 203, Fish, Acute Toxicity Test (rev. 1992). The study was conducted to fulfill the following data requirements: U.S. EPA FIFRA § 72-1; Canadian PMRA Ref.: DACO 9.5.2.1; and EU Council Directive 91/414/EEC (1991). The conditions of the test were evaluated according to OCSPP 850.1075 and OECD 203 guidelines. The following deviations were noted:

1. The age of the fish was not reported, although the reported length and weight indicate that they were juveniles.
2. The source of the dilution water was not reported.
3. The test temperatures ranged from 11.7 to 12.2°C; OECD guidelines suggest a range of 13-17°C. However, the test temperatures fell within the range of 12 ± 2°C as suggested by OCSPP guidelines.
4. Only a single aquarium with 10 fish was tested for each control and treatment group; OCSPP guidelines suggest that 2 replicates per test concentration are preferred to provide a stronger statistical baseline. OECD guidelines do not address replication.
5. Physico-chemical properties of the test material other than solubility were not reported; OECD and OCSPP guidelines suggest such information should be reported.
6. The length of the fish at test initiation was 5.5 ± 0.6 cm; OECD guidelines suggest that the length of the fish should be 2 ± 1.0 cm. OCSPP guidance only suggests that the length of the longest fish should not be more than twice the length of the shortest fish.
7. There was no transition period between light and dark conditions; OCSPP guidelines suggest that there be a 15 or 30 minute transition period.

These deviations do not affect the scientific soundness of this study.

COMPLIANCE: Statements of Data Confidentiality, GLP, and Quality Assurance were provided. The study was conducted in compliance with the current OECD Principles of Good Laboratory Practice and with the current Principles of GLP according to Annex 1 of the German chemical law (ChemG) dated June 20, 2002, except for water quality measurements of the deionized water for residues and contaminants. The study also meets the requirements of the USEPA FIFRA GLP standards (40 CFR Part 160) and the GLP standards of the JMAFF (11 Nousan No. 6283; October 1999), with the exception that recognized differences exist between the OECD principles/standards and those of FIFRA and JMAFF.

A. MATERIALS:

Data Evaluation Report on the Acute Toxicity of Fluopyram & Trifloxystrobin SC 500 (250+250) G (AIs: fluopyram and trifloxystrobin) to Fish (*Oncorhynchus mykiss*)

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1. Test material	**AE C656948 &Trifloxystrobin SC 250 + 250 G (AIs: fluopyram and trifloxystrobin)**
Description:	White suspension
Lot No./Batch No. :	2007-000441 (batch ID)
Purity:	21.4% fluopyram; 21.6% trifloxystrobin
Stability of compound under test conditions:	The test material was only analyzed for the presence of fluopyram. Day 0 recoveries ranged from 95 to 110% of nominal test levels, and Day 4 recoveries (two lowest test levels only) ranged from 99 to 102% of nominal. Fluopyram appeared very stable under the test conditions.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals:	The test material was stored at room temperature.

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Physicochemical properties of AE C656948 &Trifloxystrobin SC 250 + 250 G (AIs: fluopyram and trifloxystrobin).

Parameter	Values	Comments
Water solubility at 20EC	Fluopyram: 16 mg/L Trifloxystrobin: <0.61 mg/L	At pH 7. In Milli-Q water at 25°C.
Vapor pressure	**Not reported**	
UV absorption	**Not reported**	
pKa	**Not reported**	
Kow	**Not reported**	

2. Test organism:

Species: Rainbow trout (*Oncorhynchus mykiss*) *EPA recommends a cold water species (preferably rainbow trout Oncorhynchus mykiss) and a warm water species (preferably bluegill sunfish Lepomis macrochirus). OECD recommends choice of species at discretion of testing laboratory.*

Age at test initiation: Not reported

Weight at study initiation: 1.6 ± 0.6 g *EPA recommends: mean 0.5 - 5 g.*

Length at study initiation: 5.5 ± 0.6 cm *EPA recommends: Longest not > 2x shortest; OECD recommends 2.0 ∀ 1.0 cm for bluegill and 5.0 ∀ 1.0 cm for rainbow trout*

Source: *EPA recommends that all organisms be from the same source*

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study A range-finding study was conducted but not reported.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation		

Parameter	Details	Remarks *Criteria*
Period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	14 days Photoperiod was the same; could not determine if temperature and water were the same Fish were fed daily with commercial trout food (Brutfutter Ecostart 17, BioMar, Denmark). Fish were not fed 48 hours before and during the study. In the 48-hour period before testing, <5% of the fish died. All unsuitable fish were eliminated prior to the assignment of test groups.	*The recommended acclimation period is a minimum of 14 days; OECD guideline recommends a minimum of 12 days. Pretest mortality should be < 3% 48 h. prior to testing. OECD pretest mortality criteria: >10% = rejection of entire batch; ≥ 5 and ≤ 10% = continued acclimation for 7 days; <5% = acceptable.*
Duration of the test	96 hours	*The recommended test duration is 96 hours.*
<u>Test condition</u> Static/flow-through Type of dilution system - for flow-through method. Renewal rate for static renewal	 Static N/A	*A reproducible supply of toxicant is recommended. Consistent flow rate is usually 5-10 vol/24 hours; meter systems should be calibrated before and after study and checked twice daily during test period.*
Aeration, if any	None	*Aeration is not recommended; OECD guideline recommends aeration. If aeration is necessary, test solutions must be analyzed periodically to verify exposure.*

Parameter	Details	Remarks *Criteria*
Test vessel Material: (glass/stainless steel) Size: Fill volume:	 Glass 32 x 36 x 38 cm (l x d x h) 40 L	 *Test vessel size is usually 19 L (5 gal) or 30 x 60 x 30 cm.* *Fill volume is usually 15-30 L of solution.*
Source of dilution water Quality:	Demineralized water was used for testing, and the source was not reported. Salt stock solutions were added to the water to prepare reconstituted water. This water was aerated to reach oxygen saturation.	*Recommended source of dilution water is soft, reconstituted water or water from a natural source. EPA does not recommend the use of dechlorinated tap water; however, its use may be supportable if the biological responses for the organisms and chemical analyses of residual chlorine meet conditions in the Agency=s 850.1010 guidelines for dilution water (http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1010.pdf) Dilution water should be intensely aerated before the study. OECD permits dechlorinated tap water.*

Data Evaluation Report on the Acute Toxicity of Fluopyram & Trifloxystrobin SC 500 (250+250) G (AIs: fluopyram and trifloxystrobin) to Fish (*Oncorhynchus mykiss*)

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Parameter	Details	Remarks *Criteria*
Water parameters: Hardness pH Dissolved oxygen Total Organic carbon Particulate Matter Metals Pesticides Chlorine Temperature {Salinity for marine or estuarine species} Intervals of water quality measurement	 40-60 mg/L as $CaCO_3$ 6.8-7.2 88-100% O_2 <2 mg/L <5 mg/L Not detected Not detected <0.01 mg/L 11.7-12.2°C N/A Temperature, dissolved oxygen and pH were measured daily.	 *Hardness:* *EPA recommends 40 - 48 mg/L as $CaCO_3$ (OECD recommends 10 - 250 mg/L)* *pH:* *EPA recommends 7.2 - 7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8); (OECD recommends pH 6.0 - 8.5)* *Dissolved Oxygen:* *EPA recommends: Static: ∃ 60% during first 48 hrs and ∃ 40% during second 48 hrs; flow-through: ∃ 60%; (OECD guideline recommends at least 80% saturation value).* *Temperature:* *EPA recommends 12 EC for coldwater species, 17 or 22 EC for warmwater species, and 22 ± 1 EC for estuarine/marine organisms. (OECD recommends 21 - 25°C for bluegill and 13 - 17°C for rainbow trout).* *Salinity:* *EPA recommends 30-34‰ (parts per thousand) for marine, 10-17‰ for estuarine fish, weekly range < 6‰.* *Water quality should be measured at beginning of test and every 48 hours.*
Number of replicates/groups: control: solvent control: treated ones:	 1 N/A 1	 *Recommended number of replicates include a control and five treatment levels. Each concentration should be 60% of the next highest concentration; concentrations should be in a geometric series.*
Number of organisms per replicate /groups: control: solvent control: treated ones:	 10 N/A 10	 *Number of organisms per replicate should be ≥ 10/concentration; OECD guideline recommends at least 7 fish/concentration.*

Parameter	Details	Remarks *Criteria*
Biomass loading rate	0.40 g fish/L	*Recommended static conditions are ≤ 0.8 g/L at ≤ 17°C and ≤ 0.5 g/L at > 17°C. Recommended flow-through conditions are ≤ 1 g/L/day. OECD recommends a maximum of 1 g fish/L for static and semi-static, while higher rates are recommended for flow-through.*
Test concentrations: Nominal (formulation): Nominal (AI: fluopyram): Mean-measured (AI: fluopyram): Nominal (AI: trifoxystrobin):	 0 (negative control), 31.3, 62.5, 125, 250, and 500 µg form/L 0 (negative control), 6.70, 13.4, 26.8, 53.5, and 107 µg fluopyram/L <0.521 (<LSC; negative control), 7.26, 13.6, 26.1, 53.1, and 103 µg fluopyram/L 0 (negative control), 6.76, 13.5, 27.0, 54.0, and 108 µg trifloxystrobin/L	LSC: lowest standard concentration
Solvent (type, percentage, if used)	N/A	*The solvent should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests; OECD recommends that the solvent not exceed 100 mg/L.*
Lighting	16L:8D No transition period	*The recommended photo period is 16 hours of light and 8 hours of dark with a 15-30 minute transition period. OECD recommends a photo period of 12 -16 hours.*
Feeding	Fish were not fed during the test	*Fish should not feed during the study.*
Recovery of chemical Frequency of determination Level of quantization	 Analytical determinations of fluopyram concentrations were conducted in the test media at test initiation, at 24 and 48 hours, and at test termination. Only select test levels were analyzed at day 1, 2, and 4. Not reported	LSC: lowest standard concentration used in lieu of the LOD or LOQ

Parameter	Details	Remarks *Criteria*
Level of detection	Not reported	
Positive control {if used, indicate the chemical and concentrations}	None	
Other parameters, if any	None	

2. Observations:

Table 2: Observations

Parameter	Details	Remarks *Criteria*
Parameters measured including the sublethal effects/toxicity symptoms	-mortality -intoxication	
Observation intervals	4, 24, 48, 72, and 96 hours.	
		Observation intervals should be a minimum of every 24 hours.
Were raw data included?	Yes	
Other observations, if any	None	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

Mortality was 0% in the control and nominal 31.3 and 62.5 µg form/L treatment levels. Mortality was 100% in the 125, 250, and 500 µg form/L treatment levels.
EPA/OECD require pretreatment control mortality < 10%.. EPA requires that control or solvent mortality not exceed 10%. OECD requires that maximum-allowable control or solvent control mortality is 10% (or 1 mortality if 7 to 10 control fish are used) for a 96-h period of testing.

Table 3: Effect of AE C656948 &Trifloxystrobin SC 250 + 250 G (AIs: fluopyram and trifloxystrobin) on Mortality of Rainbow Trout (*Oncorhynchus mykiss*).

Treatment (µg form/L) Nominal	No. of fish at start of study	Observation period					
		Day 4		Day 24		Day 96	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Control	10	0	0	0	0	0	0
31.3	10	0	0	0	0	0	0
62.5	10	0	0	0	0	0	0
125	10	0	0	4	40	10	100
250	10	3	30	10	100	10	100
500	10	10	100	10	100	10	100
NOAEC	62.5 µg form/L						
LC_{50} (95% confidence interval)	N/A			133 (ND)		91 (ND)	
Positive control, if used mortality: LC_{50}:	N/A						

ND - not determined due to mathematical reasons

B. NON-LETHAL TOXICITY ENDPOINTS:

Signs of intoxication included inactivity/low activity, labored respiration, lying on the bottom of the aquarium, weak coloration, lying on their sides or backs, or loss of equilibrium with lateral deviation from normal orientation. Fish in the control and nominal 31.3 and 62.5 µg form/L treatment levels did not shows signs of abnormality. At 24 hours, all of the surviving fish in the 125 µg form/L treatment group showed signs of toxicity. At 4 hours, all of the surviving fish in the 500 µg form/L treatment group showed signs of toxicity.

Table 4: Sub-lethal Effect of AE C656948 &Trifloxystrobin SC 250 + 250 G (AIs: fluopyram and trifloxystrobin) on Rainbow Trout (*Oncorhynchus mykiss*).

Treatment (µg form/L) Nominal	Observation period				
	4 hours	24 hours	48 hours	72 hours	96 hours
	No. affected	No. affected	No. affected	No. affected	No. affected
Control	10- N	10- N	10- N	10- N	10- N
31.3	10- N	10- N	10- N	10- N	10- N
62.5	10- N	10- N	10- N	10- N	10- N
125	3- N 5- BO, SR, AT, AP 2- AP, AT	2- HF, SR, TS, AT 4- BO, SR, AT, KR*, HF	10- TF	--	--
250	7- BO, SR, AT, AP 3- TF	10- TF	--	--	--
500	10- TF	10- TF	--	--	--
NOAEC	Not reported				
LOAEC	Not reported				
EC_{50}	Not reported				
Positive control, if used % sublethal effect: EC_{50}:	N/A				

* The behavioral observation 'KR' could not be found in the list of meanings of the codes used for denoting the different types of intoxication. The code 'DF' was listed, but was not found in the table of mortality and behavioral observations. It is possible that KR and DF are the same, but this could not be confirmed.

N – Normal AP – inactive AT – labored respiration
BO – remained at bottom DF – dark in coloration* SR – laid on their sides or backs
TF – dead HF- weak coloration
TS – loss of equilibrium with lateral deviation
-- complete mortality precluded ability to make behavioral observations

C. REPORTED STATISTICS:

When possible the mortality data was analyzed for each 24-hour interval for determination of the LC_{50} values and the 95% confidence intervals using the moving average, logit or probit analysis. The appropriate method was determined according to the data characteristics. If case responses were 0% or 100%, the values were transformed into 0.10% and 99.9% to reach a probit/logit analysis. Analyses were conducted using ToxRat version 2.09.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer visually determined the toxicity values for sublethal effects data. Mortality data
were analyzed using the binomial test in the statistical program Toxanal. The reviewer analyzed the data in

terms of nominal concentrations of the formulation and the active ingredient trifloxystrobin, and mean-measured concentrations of fluopyram.

Formulation (nominal)
LC_{50}: 88.4 µg form/L 95% C.I.: 62.5 to 125 µg form/L
NOAEC: 62.5 µg form/L (based on mortality and sublethal effects)
Probit Slope: N/A 95% C.I.: N/A

Fluopyram (mean-measured)
LC_{50}: 18.8 µg fluopyram/L 95% C.I.: 13.6 to 26.1 µg fluopyram /L
NOAEC: 13.6 µg fluopyram/L (based on mortality and sublethal effects)
Probit Slope: N/A 95% C.I.: N/A

Trifloxystrobin (nominal)
LC_{50}: 19.1 µg trifloxystrobin/L 95% C.I.: 13.5 to 27.0 µg trifloxystrobin/L
NOAEC: 13.5 µg trifloxystrobin/L (based on mortality and sublethal effects)
Probit Slope: N/A 95% C.I.: N/A

E. STUDY DEFICIENCIES:

There were no study deficiencies.

F. REVIEWER'S COMMENTS:

The reviewer's and the study author's results were very similar. The reviewer's LC_{50} value was slightly lower and 95% confidence limits were obtained; therefore, the reviewer's results are presented in the Executive Summary and Conclusions sections of this DER.

The use of time weighted averages (TWA) were not necessary in this study, as recoveries were very high (ranging from 95 to 110% at test initiation and 99 to 102% at test termination) and measured concentrations did not experience any random fluctuations throughout the test. Therefore, mean-measured concentrations were used.

The study author did not analyze test solutions for each day of the test duration, and also did not analyze all test solutions except for at time 0. This resulted in the highest treatment level only being measured at time 0 (presumably because all fish had died by 24 hours), so this was the only value used for the reported measured concentration at that particular test level.

The test was conducted between September 17, 2007 and September 21, 2007.

G. CONCLUSIONS:

The study is scientifically sound and is classified as acceptable. The NOAEC (based on mortality and sublethal effects) and LC_{50} values were 62.5 and 88.4 µg form/L, which were equivalent to 13.6 and 18.8 µg fluopyram/L and 13.5 and 19.1 µg trifloxystrobin/L. Based on the results of this study, **AE C656948 &Trifloxystrobin SC 250 + 250 G** would be classified as very highly toxic to rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis.

Formulation (nominal)
LC_{50}: 88.4 µg form/L 95% C.I.: 62.5 to 125 µg form/L
NOAEC: 62.5 µg form/L (based on mortality and sublethal effects)
Probit Slope: N/A 95% C.I.: N/A

Fluopyram (mean-measured)
LC_{50}: 18.8 µg fluopyram/L 95% C.I.: 13.6 to 26.1 µg fluopyram /L
NOAEC: 13.6 µg fluopyram/L (based on mortality and sublethal effects)
Probit Slope: N/A 95% C.I.: N/A

Trifloxystrobin (nominal)
LC_{50}: 19.1 µg trifloxystrobin/L 95% C.I.: 13.5 to 27.0 µg trifloxystrobin/L
NOAEC: 13.5 µg trifloxystrobin/L (based on mortality and sublethal effects)
Probit Slope: N/A 95% C.I.: N/A

Sublethal effects: inactivity/low activity, labored respiration, lying on the bottom of the aquarium, weak coloration, lying on their sides or backs, and loss of equilibrium with lateral deviation from normal orientation

III. REFERENCES:

Brauhn, J.L. Schoettger, R.A., "Acquisition and Culture of Research Fish: Rainbow Trout, Fathead Minnows, Channel Catfish and Bluegill Sunfish". Environmental Protection Agency, Ecological Research Series EPA-660/3-75-011, May 1975.

ToxRat version 2.09 (release 2006-11-08) produced by ToxRat Solutions GmbH, 52477 Alsdorf, Germany.

Stephan, C.E., 1977, Methods for Calculating an LC50. In: Aquatic Toxicology and Hazard Evaluation, ASTM STP 634. F.L. Mayer and J.L. Hamelink, eds. American Society for Testing and Materials, Philadelphia, PA. 65-84.

ASTM Standard E 729-1988, Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. Philadelphia, PA.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL ANALYSIS:

Moncie Wright Fluopyram & Trifloxystrobin Formulation Acute

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
500	10	10	100	9.765625E-02
250	10	10	100	9.765625E-02
125	10	10	100	9.765625E-02
62.5	10	0	0	9.765625E-02
31.3	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 62.5 AND 125 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 88.38834

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE
NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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Moncie Wright Fluopyram Acute

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
103	10	10	100	9.765625E-02
53.1	10	10	100	9.765625E-02
26.1	10	10	100	9.765625E-02
13.6	10	0	0	9.765625E-02
7.26	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 13.6 AND 26.1 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 18.84038

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE
NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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